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Progression to an Androgen-Independent Disease

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PROGRESS REPORT

Project 1: *The role of the TGF β pathway in prostate cancer progression to an androgen-independent disease..*

Two manuscripts are now being written. The following paragraphs summerize these papers.

1) "Disruption of the TGF- Pathway in Transgenic Mice Induces Pre-neoplastic Lesions and Delays Castration-Induced Prostatic Regression"

The TGF- β pathway has been associated with two significant, interrelated events in prostate biology: tumorigenesis and castration-induced regression. Early reports have noted increased expression of TGF- β 1 and loss of TGF- β receptor type 1 (T β RI) and II (T β RII) expression with increasing cancer grade in human prostate cancer (PCa). Although the TGF- β superfamily has been implicated in the progression of human PCa, it's role in the development or progression of this disease has not been addressed in transgenic animal models. We have generated transgenic mice that express a metallothionein (MT) promoter driven truncated T β RII which results in a dominant negative mutant (DNIIR) that can form a heteromeric complex with the endogenous T β RI. The prostates from two different MT-DNIIR founder lines (MTR4 and MTR27H) showed transgene expression by *in situ* hybridization in all lobes of the prostate along with histopathological presence of mouse prostatic intraepithelial neoplasia (mPIN) beginning around 12 weeks of age with only a singular local invasion intraductal carcinoma occurring at 33 weeks. The lesions induced by disruption of TGF β signaling resemble those found in human PCa. Currently, human PCa is treated by androgen-ablation to induce prostate regression. TGF- β is negatively regulated by androgens and has been implicated as a mediator of castration-induced cell death in the prostate. To determine the effects of loss of TGF- β signaling in the regressing prostate, MTR4 and MTR27H mice were castrated and their prostates were examined grossly and immunohistologically with Proliferating Nuclear Cell Antigen. Compared with non-transgenic mice, the MT-DNIIR mice showed pre-neoplastic lesions and delayed prostatic regression. Although the origin of androgen independent prostate cancer in humans remains unknown, these data suggest that disruption of the TGF- β pathway may be a contributing event in the development of androgen independent disease through the prevention of complete prostatic regression after androgen ablation.

2) *Loss of TGF- β signaling alters gene expression patterns and accelerates prostate tumorigenesis in transgenic mice.*

Prostate cancer is the most commonly occurring cancer and the second leading cause of cancer death in American men. In human prostate cancers, loss of functional Transforming Growth Factor- (TGF-) receptor type II (T RII) has been associated with higher tumor grade and poor prognosis. To elucidate the importance of this correlation, three transgenic mouse lines (MT-DNIIR, LPB-Tag, and Bigenic) were created to provide a mammalian *in vivo* model system to study prostate tumor development and progression. MT-DNIIR express a truncated T RII driven by the metallothionein promoter to create a dominant negative phenotype in prostatic epithelial cells. LPB-Tag express the oncogenic SV40 large T antigen in prostatic epithelial cells using the long probasin promoter². Bigenic mice were generated by crossbreeding the MT-DNIIR and LPB-Tag lines to express both transgenes. MT-DNIIR develop prostatic lesions

comparable to human high-grade prostatic intraepithelial neoplasia (HGPIN) but rarely prostate cancer. LPB-Tag develop HGPIN that progresses to locally invasive carcinoma by 20 weeks of age. Bigenic offspring aged 12-23 weeks were studied to determine the effect of loss of TGF-signaling on prostate cancer progression. While age-matched MT-DNIIR and LPB-Tag develop only HGPIN at comparable time points, Bigenic mice develop invasive prostate cancer with both glandular and neuroendocrine differentiation at 16 weeks. Additionally, bigenic mice develop metastatic lesions – especially to the liver and lung – that are not observed in age-matched LPB-Tag mice. The bigenic mice have a statistically significant increase in metastasis and metastatic tumor burden.

To identify the gene changes that accompany increase metastatic burden, microarray analyses of bigenic versus LPB-Tag dorsolateral prostate has been performed. Samples at 12, 16, 18.5, 20, and 23 weeks showed a total of 996 out of 5000 genes examined with differential fold expression of either 1.5 or 0.7. Using the top 50 differentially expressed genes, the mice within each transgenic age group clustered together upon pair-wise comparison, showing there are age-dependent differential gene expression patterns. The top 50 genes were selected based on four statistical methods: Significance Analysis of Microarrays, weighted gene analysis, mutual-information scoring, and permutation t-test. Comparing the early and late ages (by the agglomerative hierarchical clustering algorithm) revealed that the early ages (12,16 weeks) and late ages (20,23 weeks) clustered together as distinct groups. The microarray data correlated with the histological results showing a significant difference between Bigenic and LPB-Tag mice starting at 16 weeks. The temporal patterns of expression will allow us to identify the TGF-mediated pathways contributing to prostate cancer progression and potential therapeutic targets.

Project 2: *Tumorigenic effects of partial versus complete ablation of the TGF β type II receptor in prostatic epithelial cells.* The first Task of this grant has been to follow the expression pattern of the TGF β type II receptor during the development of the prostate. It is well established that TGF β is involved in the differentiation of the luminal epithelial cell and in the regression of the prostate after castration. Multiple attempts with different techniques have been used to measure the type II receptor levels; however, the levels of the type II receptor appear to be so low that accurate measurements have been very difficult. A two month exposure via *in situ* hybridization will be developed in May which should detect cellular localization of the receptor. To parallel studies done with the MT-DNIIR transgenic mice (where the MT promoter is not prostate or cell type specific), ARR₂PB-DNIIR transgenic mice were made such that prostatic epithelial cell specific expression could be targeted. Characterization of three lines with this construct (and eleven with the LPB promoter) reveal no expression of the DNIIR in mature mice. Prostatic histology looks normal even on aging of the mice. However, differentiated function is altered such that prostatic cells are not producing the wild type levels of probasin. Rather, probasin levels have decreased 100-fold in the transgenic mice. Within the rodent prostate, two different types of dorsolateral epithelial cells have been reported that are distinct by histology and by the expression profiles of probasin^{3, 4}. One cell population expresses very low levels of probasin. Since expression of the DNIIR in the epithelial cell during development would block TGF β signals that are involved in differentiation of the prostate such that differentiated gene expression may be altered. Targeting the DNIIR with a prostate-specific promoter like probasin downregulates the expression of the probasin gene and thus the promoter construct. This results in a selection that allows only cells that express very low levels of probasin, and thus the PB promoter, to exist. The MT promoter works since it continues expression in response to zinc concentrations and not

to the selection of differentiated function by the cells. The current approach of using the cross of the ARR₂PB-Cre mice⁵ with *Tgrbr2*^{floxE2} mice is the most important since it removes the type II receptor gene from the cell. This will effect differentiation, but unlike the ARR₂PB-DNIIR, continued control by the probasin promoter is not necessary for an effect. Once the Cre enzyme is expressed, floxing of the type II receptor occurs and is non-reversible. Thus, loss of the type II receptor gene in this approach provides an extremely reliable method to remove the gene and study the consequences on prostate development and tumor development.

Project 3: *Tumorigenic effect of TGF in mouse prostatic epithelial cells and the therapeutic efficacy of combined blockade of EGF receptor and TGF cleavage in mouse prostate cancer.*

TGF α , one of the ligands of the Epidermal Growth Factor Receptor (EGFR) is overproduced in human prostate cancer. Using a mouse model that over-expresses TGF α , we have shown that this expression promotes prostatic intraepithelial neoplasia (PIN) in these animals. We have shown that these lesions are concurrent with hyperphosphorylation of the EGFR as well as both MAP kinase and AKT, effector molecules thought to be down stream of TGF α signaling and involved in mediating the increased proliferation and decreased apoptosis which we also observe in the prostate of the transgenic animals. In addition, we have shown that combining over-expression of TGF α to stimulate the EGFR axis while simultaneously inhibiting the TGF β axis via a dominant negative type II receptor results in a higher-grade lesion early on than either transgene promotes alone. The EGFR axis lends itself to pharmacological manipulation and or targeting of the EGFR kinase activity show a modest effect on prostate growth in a mouse model of prostate cancer. Our findings thus far indicate that TGF α plays a role in growth and development of prostate cancer but that it is insufficient to drive the development of the disease on its own. Two manuscripts are in preparation.

KEY RESEARCH ACCOMPLISHMENTS

Project 1: *The role of the TGF β pathway in prostate cancer progression to an androgen-independent disease.*

Task I: Characterization of MT-DNIIR-27 and MT-DNIIR-4 mice. Task completed, paper being prepared.

- With aging, the MT-DNIIR-27 and MT-DNIIR-4 transgenic mice show the most significant changes in the dorsolateral prostate which include the development of high grade prostatic intraepithelial neoplasia (HGPIN).
- In one MT-DNIIR-4 transgenic mouse, invasive prostate cancer developed at 33 weeks of age. However, due to skeletal defects that these mice also develop after 30 weeks of age, we are not able to maintain the animals past this time. Therefore, all long term studies are now being carried out with the MT-DNIIR-27 transgenic line.
- With androgen removal (castration) the prostate of the MT-DNIIR mice regress at a slower rate. Slowing down prostate regression by the loss of the TGF β signaling pathway suggest that a loss of this pathway in prostate cancer can play a role in tumor progression

Task II: MT-DNIIR x LPB-Tag transgenic lines. Studies Completed on the DNIIR cross with two different lines of LPB-Tag: 12T-7f (PIN and invasive cancer model, rare metastasis) and 12T-10 (Neuroendocrine, NE, cancer that with NE metastasis) LPB-Tag tumors.

MT-DNIIR x 12T-7f Cross

- The incidence of primary prostate cancer is now being analyzed in a blinded study by Scott Sheppall coming MT-DNIIR x 12T-7f to 12T-7f alone.
- The incidence of metastatic NE cancer increases when the MT-DNIIR gene is expressed in the 12T-7f and is statistically significant ($p < 0.05$) ..

MT-DNIIR x 12T-10 Cross

- The primary NE prostate cancer still occurs in the cross as seen in the 12T-10 alone ⁶.
- The incidence of primary NE prostate cancer is the same between the cross and 12T-10 alone.
- The incidence of metastatic NE cancer increases in the cross and is statistically significant ($p < 0.05$).

Conclusion: The loss of the TGF β pathway does appears to contribute to the spread of prostate cancer.

Task III: Progression after androgen ablation in the LPB-Tag mice. Animal study completed. Immunohistological studies remain to be completed.

- Animals have been castrated, tumors regressed and 74% regrowth following 2-6 months.
- Pathological analysis shows that the regrowing tumors are PIN, sarcomas, and NE cancers.
- Metastatic tumors appear frequently in the castrated mice, mostly as NE cancers.

Task IV: Progression after androgen ablation in the MT-DNIIR x LPB-Tag Mice. These studies have started but have become problematic. After castration of the MT-DNIIR x 12T-7f, mice frequently die. Task II has taught us that the cross develops a more aggressive cancer. Publish work indicates that the ligand (DHT) bound AR can bind to active Smad3 to block its activity ⁷. Task I has taught us that the prostate of MT-DNIIR mice regresses slowly. Also, the NE cancer that develops in the 12T-10 starts as a PIN lesion that is AR positive and chromogranin A positive (a NE cell marker) but progresses to invasive and then metastatic NE cancer that is AR negative. The normal route of dedifferentiation of NE cells is that they lose AR. Taken together, this data suggest that castration of the MT-DNIIR x 12T-7f could actually accelerate the dedifferentiation of the NE cancer resulting in death. We are now investigating this possibility.

Project 2: *Tumorigenic effects of partial versus complete ablation of the TGF β type II receptor in prostatic epithelial cells.*

Task I: Characterize T β RI and T β RII expression during prostate development in the mouse.

Three different methods were used in effort to detect TGF β Type II Receptor in mouse prostates of 1,3,5,6 weeks old animals.

- The first try was the immunostaining using new to us Rabbit Polyclonal Antibody (Gift from Antonia Teresita) to TGF β RII.
 - Problems with specificity could not be overcome.
- Real Time PCR using RNA isolated from above time points and primers to TGF β RII, done in collaboration with Scott Shappell laboratory.
 - Technical difficulties using this method did not led to any results.
- In Situ Hybridization on paraffin sections of prostates. The 35 S labeled Exon 2 and 3 of TGF β RII was hybridized to 1,3,5,6, weeks old prostate sections .
 - The slides are currently being exposed to research emulsion (for 2 month) and will be developed on May 20th. The probe for this in situ was carefully designed, trying to exclude previous problems such as CG rich regions and 3' overhang ends of template during digestion.

Task II: Disrupt the TGF- β pathway specifically in epithelium with the ARR₂PB-DNIIR transgene.

- We have made eleven transgenic lines with the Large PB promoter construct and three lines with the new ARR₂PB promoter to target prostate specific expression of the DNIIR to the prostate of transgenic mice. None the lines express the DNIIR gene.
- Two types of luminal epithelial cells exist in the rodent prostate, one type expresses high levels of probasin and the second type very low levels of probasin. By RT-PCR, we have confirmed that in the ARR₂PB-DNIIR transgenic mice, 100-fold lower levels of probasin exist compared to non-transgenic mice.

Conclusion: Expression of the DNIIR selects during prostatic development and growth for a population of luminal epithelial cells that have a differentiation pattern that does not express probasin. Since these cells express very low levels of probasin, the probasin promoter construct we used are also downregulated such that DNIIR is no longer expressed. Thus, the MT-DNIIR is the only construct that will work.

Task III: Create and cross breed ARR₂PB-Cre mice with *Tgrbr2*^{fl_{oxE2}} mice for complete abrogation of TGF- β signaling.

- A line of ARR₂PB-Cre^{+/+} mice was generated in the laboratory of Dr. Pradim Roy-Burman.
- This line of ARR₂PB-Cre^{+/+} mice is presently being crossbred with mice carrying the *Tbrbr2*^{fl_{oxE2}} allele. As a preliminary experiment, 7wk prostates from animals carrying the *Tbrbr2*^{pk} allele were harvested and examined for a phenotype. Initial results indicate that 7wk prostates from these bigenic animals may exhibit some epithelial disorganization in the anterior prostate. Furthermore, Cre expression was detected in the epithelial cells of the anterior prostate only.
- Presently, ARR₂PB-Cre^{+/-}: *Tbrbr2*^{fl_{oxE2}/wt} are being crossbred with *Tbrbr2*^{fl_{oxE2}/ffl_{oxE2}} animals to generate more animals carrying the *Tbrbr2*^{pk} allele. Animals carrying the

Tbrbr2^{pk} will be evaluated for the absence of TBRII in prostatic epithelial cells at E18.5, post-natal stages of 1,3,5, and 6 weeks.

- Prostates will also be examined for the presence of PIN lesions and invasive/metastatic prostatic adenocarcinoma at 3,6,9, and 12 months of age.

Project 3: *Tumorigenic effect of TGF α in mouse prostatic epithelial cells and therapeutic efficacy of combined blockade of EGF receptor and TGF α cleavage in mouse prostate cancer.*

Task I: To develop and characterize ARR₂PB-TGF α transgenic mice and compare them to MT-TGF α mice.

- Our latest advance in constructs has replaced the LPB promoter with the ARR₂PB promoter⁸. Using this construct, four independent transgenic mouse lines have been generated carrying a ARR₂PB driven TGF α gene.
- Unfortunately, a sensitive radioimmuno assay did not detect any expression of human TGF α in the prostates of these lines.
- A blinded histological analysis of the prostate tissue found no difference between ARR₂PB-TGF α compared to non-transgenic littermates.
- Twenty male MT-TGF α mice have been generated for comparison with the ARR₂PB-TGF α mice but will now be used as a control for the mice in task II.

Similar to our results with the ARR₂PB-DNIIR, the ARR₂PB-TGF α transgenic lines do not make the transgene. Again, we believe that a selection for an epithelial population that cannot express the probasin protein and thus the probasin driven transgene has occurred. Given the lack of detectable protein expression ARR₂PB-TGF α , we do not believe comparison to MT-TGF α mice will prove informative.

Task II: To cross MT-TGF α mice to MT-DNIIR mice as well as to cross ARR₂PB-TGF α mice to ARR₂PB-DNIIR and/or ARR₂PB-CRE/*Tgfb α* ^{floxE2/floxE2} mice.

- Twenty male bigenic MT-TGF α crossed with MT-DNIIR mice have been generated, along with equal numbers of non-transgenic mice and animals with either the MT-TGF α or MT-DNIIR transgenes alone. Five of each group of mice were sacrificed at 15, 22, 32, and 38 weeks of age.
- The bigenic mice were found to develop high-grade PIN lesions earlier (15 weeks) than animals with only one of the transgenes (22 weeks). These lesions did not progress beyond PIN by 38 weeks of age. These animals did, however, develop primary liver tumors by 38 weeks of age, much earlier than tumors have been seen to develop in MT-TGF α mice, indicating a synergistic relationship between the two transgenes.
- The non-transgenic animals were found to be free of prostatic lesions while transgenic animals showed PIN like lesions.
- The bigenic animals consistently showed lesions of a higher grade than those of animals with only one of the transgenes. The most significant lesions were found in the anterior and dorsal lobes.
- Histological analysis indicates that the transgenic mice have hyperphosphorylated MAP kinase and AKT and both an increase in epithelial proliferation and a decrease in apoptosis. Further analysis is in progress to determine why prostate lesions do not

progress beyond the PIN stage, with preliminary results suggesting down regulation of the AKT pathway may be involved.

- Additional experiments have been conducted to determine the interaction of EGFR axis the androgen stimulus axis. MT-TGF α and non-transgenic controls were castrated and their prostate tissue analyzed. Preliminary results indicate that the MT-TGF α transgene retards the rate of prostate regression by promoting continued proliferation after androgen withdrawal and reducing the level of apoptosis in the tissue.
- As the ARR₂PB-TGF α and ARR₂PB-DNIIR lines did not produce protein, they were not crossed.

Task III: To treat mouse prostate tumors with EGFR tyrosine kinase inhibitor and/or selective TACE inhibitor.

- Given that the MT-TGF α line did not develop cancer, the LPB-Tag line was utilized and animals were treated with the EKI-785 compound from Wyeth-Ayerst.
- A small decrease in gross weight of the dorsolateral lobe of the treated animals was detected when compared to the untreated controls.
- Molecular and histological analysis of tissues from these animals is underway to determine the effects of inhibiting the EGFR kinase activity.

CORE: Pathology Core Laboratory and Provision of Basic Histopathology Support:

- As describe last year, equipment has been purchased for the Pathology Core. It now services all the individual projects. The following techniques are provided.

Adjuvative diagnostic techniques:

- Establishment of immunohistochemical protocols and application to various models, supplementing immunostaining assays performed by individual labs, including:
- General/model characterization: Pan cyto-keratin, High molecular weight cytokeratin, CK5, PCNA, Apo-tag, AR, Chromogranin, CD31 (including on frozen sections)
- Antibody assays for Shappell Mouse-based research: 8-lipoxygenase, platelet 12-lipoxygenase, leukocyte 12-lipoxygenase, cyclooxygenase-2
- Antibodies currently being investigated/validated: Laminin, N-cadherin, E-cadherin, Beta-catenin.
- Performance of ultra-structural studies on DLP/VP on LPB-Tag 12T-7f x MT-DNIIR mouse.
- Establishment of quantitative Real Time RT-PCR assays on Roche LightCycler system, utilizing cDNA standard curves with cloned templates and

cDNA binding fluorescent probe SYBR green or oligo specific hybridization probes.

REPORTABLE OUTCOMES: The reportable outcomes of the Vanderbilt Prostate Cancer Center are divided into three sections: 1) Institutional Commitments and VPCC; 2) Research Projects, and 3) Pathology Core.

1) Institutional Commitments and VPCC: Due to the DOD funding of the Center, Vanderbilt University Medical Center, the Vanderbilt-Ingram Cancer Center, the Section of Surgical Sciences, and the Department of Urologic Surgery have made major institutional commitments that have allowed the scope of the Center to expand beyond the initial research projects.

Administration: Dr. Robert Matusik serves as the Director of the VPCC. The Center holds research meetings on the first Wednesday of the month at 1:00 pm. In addition, a second meeting is also being held the first Wednesday of the month at 5:00 pm to discuss new programs and potential grant application. At the present, there is no indication by the DOD that the PPC grants will have a renewal. Thus, we must plan for other options to continue support of the Center. Two options are now being considered: 1) SPORE on Prostate; The Specialized Programs of Research Excellences on Prostate are NCI funding programs that must have a large translational component to each grant plus COREs that would support these clinical undertakings. 2) PPG on Prostate; The Program Project Grants that will cover basic research on prostate cancer. These can be strong on basic research without a clinical program.

We held our first Retreat on April 4-5, 2002. During this time, research is progress from each project is discussed. Future direction and research experiments were planned at this retreat. The Retreat included a meeting with the Steering Committee. A main concern of the Steering Committee is the needs for continued funding. Dr. Lee, Director of the Northwestern University SPORE on Prostate and serves on the NCI SPORE review panel. Since Dr. Lee is on our DOD Steering Committee, if we apply for a SPORE, he will reclude himself from our grant on the SPORE review panel. The outlined program and information on the Steering Committee follows:

**Department of Defense
Vanderbilt Prostate Cancer Center Retreat
Department of Urologic Surgery
C-2209 MCN, Amphitheater
April 4-5, 2002**

Thursday, April 4, 2002

2:30 p.m. - 3:00 p.m.	Robert Matusik, Ph.D., Urologic Research The Role of the TGF β Pathway in Prostate Cancer Progression to an Androgen Independent Disease
3:00 p.m. - 3:30 p.m.	Hal Moses, M.D., Cell Biology Tumorigenic Effects of Partial Versus Complete Ablation of the TGF β Type II Receptor in Prostatic Epithelial Cells
3:30 p.m. - 4:00 p.m.	Shane Cutler, Ph.D., Medicine/Gastroenterology Tumorigenic Effects of TGF α in Mouse Prostate Cancer
4:10 p.m. - 4:20 p.m.	Break
4:30 p.m. - 5:30 p.m.	Chung Lee, Ph.D., Department Of Urology Northwestern University Medical Center Role of TGF-beta in Cancer Progression
5:30 p.m. - 6:00 p.m.	Randall Nixon, M.D., Urologic Surgery PSA as a Marker of Prostate Cancer Progression
6:00 p.m. - 6:30 p.m.	Business Meeting
6:00 p.m. - 6:30 p.m.	Cocktails at the University Club Main Bar
6:30 p.m. - 8:30 p.m.	Dinner at the University Club Wedgewood Room, RSVP Only

**Department of Defense
Vanderbilt Prostate Cancer Center Retreat
Department of Urologic Surgery
C-2209 MCN, Amphitheater
April 4-5, 2002**

Friday, April 5, 2002

8:30 a.m. - 9:00 a.m.	Breakfast
9:00 a.m. - 9:20 a.m.	Simon Hayward, Ph.D., Urologic Research Therapy Selection by Gene Profiling
9:20 a.m. - 9:40 a.m.	Robert Matusik, Ph.D., Urologic Research Development of Peptide Antagonist That Target Disruption of Transcription Factors That Regulate Prostate Specific Gene Expression
9:40 a.m. - 10:10 a.m.	Susan Kasper, Ph.D., Urologic Surgery Why Flutamide Becomes an Agonist in Androgen-Independent Prostate Cancer
10:10 a.m. - 10:30 a.m.	Neil Bhowmick, Ph.D. The Role of TGF-beta in the Prostate
Stroma	
10:30 a.m. - 10:45 a.m.	Coffee Break
10:45 a.m. - 11:05 a.m.	Jeff Smith, M.D., Familial Prostate Cancer
11:05 a.m. - 11:25	Jay Fowke, Ph.D. Androgen-Estrogen Interaction in Prostate Carcinogenesis
11:25 a.m. - 11:45 a.m.	Scott Shappell, M.D., Ph.D., Pathology Prospective Assessment of a Peroxisome Activated Receptor Gamma [PPAR γ] Agonist in Clinically Localized Prostate Cancer
11:45 a.m. - 12:15	Discussion
12:15 p.m.	Meeting Adjourns

The Full Steering Committee will determine operational issues and future priorities. Two full Steering Committee meeting will be held per year. The external scientific advisor will attend a yearly meeting that will coincide with the annual retreat. The Steering Committee will be composed of the following groups: The Steering Committee is composed of the following members:

Vanderbilt Prostate Cancer Center Members:

Dr. Robert J. Matusik, Prostate Cancer Center Director and Director of Urologic Research

Dr. Harold Moses, Director of Vanderbilt-Ingram Cancer Center

Dr. Robert Coffey, Director of the GI Cancer Program/ or Dr. Shane Cutler

Dr. Scott Shappell, Assistant Professor, Department of Pathology

Dr. Susan Kasper, Research Assistant Professor, Department of Urologic Surgery

Internal Advisors:

Dr. Joseph A. Smith, Chairman, Department of Urologic Surgery / or Dr. Sam Chang

Dr. Michael Cookson, Department of Urologic Surgery

Dr. Jeffrey Holt, Director of Gene Therapy Program

Dr. Simon Hayward, Department of Urologic Surgery

External Advisor:

Dr. Chung Lee, Department of Urology, Northwestern University

Consumers:

Mr. Jerry Savells

Mr. Norman Wayne Simpson

Drs. Coffey, Smith, and Holt we unable to attend the meeting. Dr. Shane Cutler served as Dr. Coffey's representative and Dr. Chang as Dr. Smith's representative. Dr. Simon Hayward was to be and External Advisor; however, he was recruited by Vanderbilt and is now an Assistant Professor and serves as an Internal Advisor. The Consumers are both past patients who were diagnosed with prostate cancer.

Budget: The budget commitments have remained the same as last year and include the following:

- The Vanderbilt University Medical Center has provided the salary for Ms. Debbie Thompson to serve as an administrative assistant to the VPCC.
- The Vanderbilt-Ingram Cancer Center has provided \$200,000/ year as support for operating expenses of the Center, for equipment, secretary (Ms. Lisa Howell) and pilot projects to expand the research endeavour.
- The Department of Urologic Surgery has provided the start-up funds to recruit Dr. Simon Hayward as a new faculty member.
- The Department of Urologic Surgery provided the funds to renovate the four offices for the Prostate Cancer Center.

- For the Urologic Oncology Fellowship Training program, Dr. Jen (MD/Ph.D) has replaced Dr. Naoya Masumori (MD/PhD) as the Urologist.

Space for VPCC: Although the planned move in date was for July 2001, the actually move in date for the Laboratories was August 2001 and the offices was November of 2001. The commitment made last year has been realized. The Floor plan was attached last year and it remains as it was attached. To review, we now have increased by 2168 sq ft. from the previous 1533 sq. ft. to new total of 3701 sq. ft. laboratory space. This includes the laboratories of Drs. Matusik, Kasper, and Hayward. These individuals and Ms. Lisa Howell, secretary, have moved into the new office space (596 sq. ft.). Two offices (136 sq. ft.) have been provided for the post-doctoral fellows. These offices will have to be shared but they will provide space for the post-doctoral fellows to work on data and write manuscripts. A conference room has been made available for routine meeting with personnel. (See last years report for detailed floor plan).

New Faculty: Last year it was reported that Drs Susan Kasper and Simon Hayward were recruited as Assistant Professors in Urologic Surgery. They both have joint appointment in Cancer Biology. Dr. Kasper started in January 2001 and Dr. Hayward in August.

- Dr. Susan Kasper has received a grant starting within the next month. This grant, entitled "Developmental Biology of the Normal Mouse Prostate" (NIH).
- Dr. Simon Hayward bring with him and has newly received the following grants entitled "Paracrine regulation of prostatic carcinogenesis" (DOD) and Hormonal Carcinogenesis in Rb-Knockout mouse" (NIH).
- Dr. Richard Roberts, M.D., Ph.D., Research Instructor and Molecular Pathology Fellow rank is now pending as Assistant Professor in Pathology in the summer of 2002. He will continue to be involved in characterization of the animal models of prostate cancer as well as a prostate cancer pathologist for the Pathology Department.

Post-doctoral Fellows

- Dr. Shane Cutler currently is working in Dr. Coffey's laboratory on Project 3 to study the role of TGF α in prostate tumor development.
- Dr. Ren Jie Jin on May 1, 2001. He is a trained Urologist from China that has also completed a Ph.D. from Seoul National University, Korea. Dr. Jin is working with Dr. Matusik's laboratory on Project 1 and on the LPB-Tag transgenic animal models. He is a new recruit to the Urology Fellowship Training Program
- Mr. Janni Mirosevich arrived September 1, 2001. Mr. Mirosevich will study gene expression on Project 1.
- Ms. Tiina Pitkänen-Arsiola arrived in July, 2001. Dr. Pitkänen-Arsiola works with Dr. Kasper's laboratory to study progression in prostate cancer from an androgen-dependent to an androgen-independent disease.

Students

- Mr. William Tu is a MD/Ph.D. student at Vanderbilt working for Dr. Matusik. (see Curriculum Vitae, Appendices). He is studying how combining the disruption of the TGF β pathway and the p53/RB pathway results in developing adenocarcinoma in Project 1.
- Miss Aparna Gupta is a Ph.D student at Vanderbilt. She has just starting working in Dr. Matusik's laboratory using MicroArray analysis of gene expression in the mouse prostate tumors.

2) Research Projects: A number of manuscripts are now in preparation on the role that TGF α and TGF β pathway plays in developing prostate cancer. A number of abstracts have been presented. Also, as a result of this work, symposium lecture at meetings have resulted.

Published Abstracts

- Tu WH, Thomas TZ, Masumori N, Tsukamoto T, Kasper S, Roberts RL, Moses HL, Shappell SB, Matusik RJ. Role of TGF- β pathway in prostate carcinogenesis. The American Urological Association 96th Annual Meeting, June 2-7, 2001. Anaheim, California.
- Robert J. Matusik, William H. Tu, Tania Z. Thomas, Naoya Masumori, Susan Kasper, Richard L. Roberts, Rosa Serra, Scott B. Shappell, and Harold L. Moses, TGF- β and Prostate Cancer. 83st Annual Meeting of The Endocrine Society, June 20-23, 2001. Denver, Colorado.
- Cutler, NS, Coffey, RJ. Overexpression of TGF α induces prostatic intraepithelial neoplasia in mice. *Modeling Human Prostate Cancer in Mice*, October 18-21, 2001. Bar Harbor, Maine.
- Robert J. Matusik, Naoya Masumori, Tania Z. Thomas, JianFeng Zheng, William H. Tu, Richard L. Roberts, Susan Kasper, and Scott Shappell, Probasin targeted Large T antigen: LADIES are not TRAMPS. *Modeling Human Prostate Cancer in Mice*, October 18-21, 2001. Bar Harbor, Maine.
- Harold L. Moses, William Tu, Tania Z. Thomas, Naoya Masumori, Susan Kasper, Richard L. Roberts, Rosa Serra, Scott B. Shappell, and Robert J. Matusik. TGF β and Prostate Cancer. *Modeling Human Prostate Cancer in Mice*, October 18-21, 2001. Bar Harbor, Maine.
- William H Tu, Yu Shyr, Paul Larsen, Shawn Levy, Susan Kasper, Harold L Moses, Richard L Roberts, Scott B Shappell, Robert J Matusik . Loss of the TGF- β signaling alters gene expression patterns and accelerates prostate tumorigenesis in transgenic mice. American Association of Cancer Research, April 6-10, 2002, San Francisco, CA.

Manuscripts

- Thomas TZ, Tu WH, Shappell S, Kasper S, Moses HL, Matusik RJ, and Serra RA. Disruption of the TGF β pathway in transgenic mice prevents castration-induced prostatic regression. In preparation.

- Tu, W, Thomas, TZ, Masumori N, Kasper, S, Shry Y, Larsen P, Levy S, Roberts RL, Shappell SB, and Moses H, and Matusik RJ. Loss of the TGF β pathway promotes prostatic metastasis. In preparation.
- Cutler, NS, Roberts, R, Shappell, S, Coffey, RJ. Overexpression of TGF α induces prostatic intraepithelial neoplasia in mice. In preparation.
- Cutler, NS, Washington, K, Deane, NG, Coffey, RJ. Interaction of the EGFR axis and TGF β axis in liver cancer. In preparation.

Symposium Lectures

- Dr. Matusik presented a Symposium lecture at the 83rd Annual Meeting of The Endocrine Society in June 2001.
- Dr. Matusik presented his work at the NIH sponsored MMHCC Workshop on *Transgenic Models for Prostate Cancer* in October 18-21, 2001.
- Dr. Moses has been invited to present his work at the NIH sponsored MMHCC Workshop on *Transgenic Models for Prostate Cancer* in October 18-21, 2001.
- Dr. Cutler's abstract was selected for oral presentation at the NIH sponsored MMHCC workshop *Modeling Human Prostate Cancer in Mice*, October 18-21, 2001.
- Dr. Shappel Chaired the Pathology Workshop held in concert with NIH sponsored MMHCC Workshop on *Transgenic Models for Prostate Cancer* in October 18-21, 2001. He has written the report on an analysis of all the mouse models for prostate cancer.

Personnel:

The personnel of the VPCC include those supported by the DOD award, institutional commitments, and individuals that may be on trainee awards [For example, Dr. Ren Jie Jin (Matusik) and Dr. Shane Culter (Coffey) are on training grants]. Listed below are only individuals supported directly by the DOD award over the fiscal year covered by this report.

PROJECT 1:

Robert J. Matusik, PhD	PI and Director
Susan Kasper, PhD	Co-Investigator
Yongqing Wang, PhD	Research Fellow
William Tu	Graduate Student

PROJECT 2:

Harold L. Moses, MD	PI
Agnieszka E. Gorska	Research Tech Senior
Mary E. Aakre	Research Tech Senior
Anna Chytil	Research Tech Senior

PROJECT 3

Robert J. Coffey, Jr, MD	PI
Galina T. Bogatcheva	Research Assistant III

PATHOLOGY CORE

Scott B. Shappell, MD,	PI
Richard L. Roberts, PhD	Research Instructor
Suzanne Manning	Research Assistant III
Cathy Hibbs-Brown	HistoTech

3) Pathology Core: Dr. Scott Shappell is director of the Pathology Core. He Chaired the NCI sponsored Mouse Models of Human Cancer Consortium (MMHCC) workshop on transgenic mouse prostate cancer models. This Workshop reported, written by Dr. Shappell, will be published this year and it will establish standards for the characterization of mouse models for prostate cancer.

CONCLUSIONS:

Substantial progress has been made on the three individual grants and in the establishment of the Pathology Core. In addition, Vanderbilt University Medical Center, Section of Surgical Sciences, Department of Urologic Surgery, and the Vanderbilt-Ingram Cancer Center have met their commitments to the DOD Center grant which are beyond the initial research projects allowing use to expand the program as a new Vanderbilt Prostate Cancer Center. The Center has held its first retreat. As a result of the retreat, a major effort is now underway to write an NIH grant for new support that would maintain the Center after the DOD funding ends.

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Ref Type: Journal (Full)
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APPENDIX

Tu WH, Thomas TZ, Masumori N, Tsukamoto T, Kasper S, Roberts RL, Moses HL, Shappell SB, Matusik RJ. Role of TGF- β pathway in prostate carcinogenesis. The American Urological Association 96th Annual Meeting, June 2-7, 2001. Anaheim, California.

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AUA 2001 Abstract Submitter

96th Annual Meeting - June 2-7, 2001

Current Abstract: 2005174 Anaheim, CA

ROLE OF TGF- β PATHWAY IN PROSTATE CARCINOGENESIS

William H Tu, Tania Z Thomas, Naoya Masumori, Nashville, TN, Taiji Tsukamoto, Sapporo, Japan, Susan Kasper, Richard L Roberts, Harold L Moses, Scott B Shappell, Robert J Matusik

Nashville, TN

Introduction and Objectives: Transgenic mice provide a mammalian in vivo system to elucidate the mechanism of prostate carcinogenesis and to serve as models for testing potential prostate cancer drug therapies. In human prostate cancers, higher tumor grade has been associated with loss of functional Transforming Growth Factor- β (TGF- β) receptor type I. To identify the role of the TGF- β pathway, the bigenic offspring from a cross of two different transgenic animal lines that develop prostatic lesions were studied.

Methods: One transgenic mouse line, 12T-7f, targets expression of the SV40 large T antigen (Tag) to the prostate using the long probasin promoter. The large T antigen has been shown to bind and inactivate two tumor suppressor genes, p53 and Rb. The second transgenic mouse line, MTR-27H, uses the metallothionein promoter to express a truncated type I receptor which results in a dominant negative mutant (DNER) that blocks the TGF- β pathway in the prostate. Both lines develop prostatic lesions comparable to human high grade prostatic intraepithelial neoplasia (HGPIN), with more pronounced epithelial proliferation and atypia in 12T-7f. Twelve male offspring aged 12-23 weeks of the bigenic transgenic mouse line (12T-7f X MTR-27H) were studied by gross, histological, and immunohistological examination (Cytokeratin, AR, Tag Chromogranin). Tissue was collected from the prostate, seminal vesicle, vas deferens, testis, bladder, bulbourethral gland, para-aortic lymph nodes, neck lymph nodes, lumbar spine, liver, lung, kidney, spleen, brain, adrenal, parotid gland, and submandibular gland.

Results: Although the age-matched transgenic mice developed only HGPIN at comparable time points, the bigenic mice developed both HGPIN and invasive prostate cancer (100 % in mice ~: 16 wks) with both glandular and neuroendocrine (N-E) differentiation. Metastatic Tag positive carcinoma, primarily with NE differentiation, was noted in para-aortic lymph nodes, bone, and viscera, including liver and lung (~: 50 % of mice 2:16 wks).

Conclusions: In contrast to the 12T-7f and DNR-U mice that develop only HGPIN at comparable time points, the bigenic offspring develop invasive carcinoma in the prostate with metastases. The TGF-P pathway and p53/RB pathways are important in prostate carcinogenesis. This study demonstrates that cross breeding transgenic mouse lines can generate new phenotypes representing improved models of human prostate cancer. These models will be helpful for the development of drug therapy in the treatment of human prostate cancer.

TGF- β AND PROSTATE CANCER. Harold L. Moses, William H. Tu, Tania Z. Thomas, Naoya Masumori, Susan Kasper, Richard L. Roberts, Rosa Serra, Scott B. Shappell, and Robert J. Matusik, Vanderbilt-Ingram Cancer Center, Nashville, TN 37232.

Significant correlative evidence has proposed a role for TGFP ligands in the development of the prostate and progression of prostate cancer (CaP). In the mature prostate, TGF-P secreted from smooth muscle stromal cells binds to TPRII expressed on the adjacent glandular epithelium and inhibits cell proliferation by inducing cell-cycle inhibitors. Castration-induced atrophy in the prostate leads to increased levels of TGFP and phosphorylated Smad. Although TGFP inhibits prostate growth, TGF-P expression is higher in proliferating human prostate carcinomas than in benign human prostate. Elevated TGFP production along with loss of TPRII expression correlates with higher tumor grade and poorer prognosis for patients with prostate cancer. It has been hypothesized that if the CaP cells are unable to respond to the inhibitory effects of TGFP I yet are over producing this potent immunosuppressor, then active expression of TGF P I could be a selective advantage. To study the effects of loss of TGFP signaling in the prostate, transgenic mice were generated that express a metallothionein (MT) promoter driven truncated TPRII dominant negative (DN) mutant (MTDNIIR). Examination of the histology of MT-DNIIR prostates revealed focal changes in prostatic morphology at approximately 12 weeks of age that are comparable to low grade prostatic intraepithelial neoplasia (LGPIN) in humans. By 16.5 weeks of age regions of high grade prostatic intraepithelial neoplasia (HGPIN) were present in all animals examined. At 33 weeks, only one mouse prostate showed a local invasion; however, these mice develop defects in the skeleton that prevents keeping them past this age. In parallel, we have generated transgenic mice that target expression of the SV40 large T antigen (Tag) to the prostate using the prostate-specific large probasin promoter (LPB). The Tag protein binds and inactivates two tumor suppressor genes, p53 and Rb, two genes that can be inactivated in late stage CaP and recent reports have identified p53 loss in some HGPIN. The LPB-Tag mice develop HGPIN by 16 weeks and some develop limited invasive cancer after 20 weeks of age. To examine how loss of TGFP signaling affects prostate tumorigenesis and cancer progression, bigenic males from the MT-DNIIR x LPB-Tag cross were studied in a time course of 12-23 weeks for gross, histological, and immunohistological characteristics. The bigenic mice developed both HGPIN and invasive prostate cancer in 100% of the animals \geq 16 wks with both glandular and neuroendocrine differentiation. Metastatic Tag positive carcinomas, primarily with NE differentiation, were noted in para-aortic lymph nodes, bone, and viscera, including liver and lung (\rightarrow 50 % of mice \geq 16 wks). Using transgenic mouse models, these studies demonstrate that the loss of the TGF-P and p53/RB pathways are important steps in HGPIN progression to prostatic adenocarcinoma (Supported by DOD Prostate Cancer Center PC992022, RO I -CA76142, and the Frances Williams Preston Laboratories of the T.J. Martell Foundation).

**OVEREXPRESSION OF TGF α INDUCES PROSTATIC
INTRAEPITHELIAL NEOPLASIA IN MICE**

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Vanderbilt University Medical Center

Nashville, TN USA

Changes in signaling through the Epidermal Growth Factor Receptor (EGFR), including an increased production of the EGFR ligand Transforming Growth Factor (α) (TGF α), have been implicated in human prostate cancer. We find that transgenic mice which overexpress TGF α under the metallothionein promoter have increased architectural complexity in all lobes of the prostate at 15 weeks of age, particularly in the anterior and dorsal lobes. The histology of these animals is similar to prostatic intraepithelial neoplasia (PIN), a precancerous condition in human prostate. A dominant-negative allele of the TGF β type II receptor expressed under the same promoter has also been shown to produce PIN-like lesions in transgenic mice. We hypothesize that the growth-promoting signal of TGF α and the growth-inhibitory signal of TGF β act in conjunction to regulate normal prostatic growth and development. Disregulation of either signal may result in abnormal growth. We further hypothesize that stimulating the EGFR while simultaneously inhibiting TGF β signaling would result in a more severe hyperplasia. To test this hypothesis, we crossed the mice with the metallothionein-driven TGF α with mice bearing a metallothionein-driven TGF β type II receptor transgene. Animals with both transgenes at 15 weeks of age exhibited a higher grade of PIN lesions with greater architectural complexity than was seen with mice bearing either transgene alone.

Probasin targeted Large T antigen: LADIES are not TRAMPS

Matusik RJ, Masumori N, Thomas T, Zhang, J-F, Tu W, Roberts, R, Kasper S, and Shappell SB*. Departments of Urologic Surgery and Pathology*, Vanderbilt Prostate Cancer Center, Vanderbilt University Medical Center, Nashville, TN 37232.

A small -426 probasin (-426PB) and a Large PB (LPB) promoter have been used to target androgen regulated transgenes to the prostate in transgenic mice. We have now developed a third small promoter, ARR2PB, that reproducibly targets high levels of transgene expression to the prostate. In the TRAMP model, the -426 PB promoter targeted the SV40 early region which includes the small and large T antigen. In the LADY model, the LPB promoter targets the large T antigen (Tag) with a deletion in the SV 40 early region that removes the expression of the small t antigen in the transgenic mouse prostate. Seven LPB-Tag transgenic lines have been established and collectively are referred to as the LADY model. A general characterization of all LPB-Tag lines including pathology, immunohistochemistry and hormonal regulation indicates that these tumors are similar to stages seen in human prostatic disease including preneoplastic lesions, local invasive carcinoma, androgen-dependent cancer and progression to androgen-independent cancer. However, one line (I 2T- 10) develops epithelial dysplasia that dedifferentiates into androgen receptor positive neuroendocrine cells which progress to androgen receptor negative metastatic neuroendocrine cancer. The six other transgenic lines (12T-1, 12T-5, 12T-7f, 12T-7s, 12T-8, 12T-11) have androgen-dependent tumors which regressed in castrated animals. These six LPB-Tag lines rarely metastasize in intact mice. Thus, they are excellent models to use in cross-breeding experiments with other transgenic line to determine the effect of a given transgene on tumor progression and metastasis. Using MRI to monitor prostate tumor size after castration of these six LPB-Tag line, we found 74% of the tumors that regressed began to regrow 2-6 months post-castration. The regrowing primary tumors varied in pathology but many developed high grade neuroendocrine carcinoma. All the neuroendocrine cancers that develop after castration or in 12T- 10 continue to express the Tag transgene in the absence of androgen and androgen receptor. This suggest that the androgen dependent probasin promoter now regulates transgene expression in an androgen independent manner.

These data demonstrate that prostate cancer in this animal model can progress through similar sequential stages that occur in human prostate cancer that include hormonal independent growth that occurs with the failure of androgen ablation therapy. This mouse model will permit an analysis of sequential genetic changes that occurs during the multistep process of carcinogenesis of the prostate. (Supported by JT Martell Foundation, R01-CA76142, DOD Prostate Cancer Center PC992022, and MMHCC UO I -CA84239)

Robert J. Matusik, Naoya Masumori, Tania Z. Thomas, JianFeng Zheng, William H. Tu, Richard L. Roberts, Susan Kasper, and Scott Shappell, Probasin targeted Large T antigen: LADIES

Matusik, Robert J DAMD17-00-1-0105

are not TRAMPS. *Modeling Human Prostate Cancer in Mice*, October 18-21, 2001. Bar Harbor, Maine.

TGF- β AND PROSTATE CANCER. Robert J. Matusik, William H. Tu, Tania Z. Thomas, Naoya Masumori, Susan Kasper, Richard L. Roberts, Rosa Serra, Scott B. Shappell, and Harold L. Moses, Department of Urologic Surgery, Vanderbilt University Medical Center, Nashville, TN 37232-2765.

Significant correlative evidence has proposed a role for TGF β ligands in the development of the prostate and progression of prostate cancer (CaP). In humans, increasing CaP grade has been correlated with increasing levels of TGF β I. As TGF β I normally inhibits prostatic cell growth, increased expression of TGF β I in CaP has presented a conundrum. It has been hypothesized that if the CaP cells are unable to respond to the inhibitory effects of TGF β I yet are over producing this potent immunosuppressor, then active expression of TGF β I could be a selective advantage. To block the TGF β in the prostate, transgenic mice were generated that express a metallothionein (MT) promoter driven truncated TPRII dominant negative (DN) mutant (MT-DNIIR). Examination of the histology of MT-DNIIR prostates revealed focal changes in prostatic morphology at approximately 12 weeks of age that are comparable to low grade prostatic intraepithelial neoplasia (LGPIN) in humans. By 16.5 weeks of age regions of high grade prostatic intraepithelial neoplasia (HGPIN) was present in all animals examined. At 33 weeks, only one mouse prostate showed a local invasion; however, these mice develop defects in the skeleton that prevents keeping them past this age. In parallel, we have developed transgenic mice that target expression of the SV40 large T antigen (Tag) to the prostate using the prostate-specific large probasin promoter (LPB). The Tag protein binds and inactivates two tumor suppressor genes, p53 and Rb, two genes that can be inactivated in late stage CaP and recent reports have identified p53 loss in some HGPIN. The LPB-Tag mice develop HGPIN by 16 weeks and some develop limited invasive cancer after 20 weeks of age. Bigenic males from the

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Symposium Lecture, Endocrine Society Meeting, Denver CO. June 20-23, 2001